Current Biology, Vol. 13, R311–R313, April 15, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00234-3

Membrane Traffic: A Glitch in the Golgi Matrix

Dispatch

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Golgins are coiled-coil proteins thought to form a matrix important for shaping and organising Golgi cisternae and directing long-range recognition events in vesicular transport. This model is brought into question by new evidence that two golgins, GM130 and golgin-84, contribute to but are not essential for protein transport and Golgi structure.

The Golgi apparatus fascinates many cell biologists, not only because of its position as an essential organelle of the secretory pathway, but also because of its complex structural organisation [1]. Golgi membranes typically form layered arrays of flattened cisternal structures adjacent to exit sites on the endoplasmic reticulum (ER) where vesicles carrying secretory and membrane cargo are produced [1,2]. Under the electron microscope, filamentous material linking Golgi cisternae can be visualised, and after detergent extraction a proteinaceous skeleton retaining the three-dimensional organisation of these Golgi cisternae remains [3]. This proteinaceous skeleton is commonly referred to as the Golgi matrix, and is known to contain a number of the elongated coiled-coil proteins of the golgin family [4,5]. We shall discuss recent evidence which has shed new light on two golgins: the first, golgin-84, is a poorly characterised integral membrane protein [6]; and the second, GM130, is a tightly attached peripheral membrane protein thought to function in docking of vesicles with cis-Golgi cisternae [7,8].

Golgin-84 is a transmembrane protein with a large cytoplasmic coiled-coil region and a transmembrane domain at its extreme carboxyl terminus identified in a yeast two-hybrid screen as an interacting partner of the phosphatidylinositol-5-phosphatase OCRL1 [6]. Together with giantin and CASP — CCAAT-displacement protein alternatively spliced product — golgin-84 forms a small family of transmembrane golgins that have the same topology and predicted coiled-coil motifs, but only display significant sequence similarity within their transmembrane domains [9]. Although golgin-84 is not a Golgi matrix protein according to the original definition of insolubility in low ionic strength buffers containing Triton X-100 [10], it has recently been shown to play a role in the general structure of the Golgi apparatus.

In a screen for Golgi proteins targeted by mitotic phosphorylation, Diao *et al.* [10] identified golgin-84 and localized it to the cis-Golgi network, inferring that golgin-84 is a potential structural or regulatory component of the cis-Golgi. They also confirmed that the Golgi stacking proteins GRASP55 and GRASP65, and the small GTPase Rab1, are mitotic phosphoproteins. Many golgins interact with members of the Rab family that regulate tethering and docking steps in membrane trafficking; for example, p115 and GM130 both interact with Rab1, the cis-Golgi-localised Rab protein essential for ER-to-Golgi transport [11,12]. Golgin-84 was also found to bind directly to Rab1, but consistent with its absence from the Golgi matrix fraction, golgin-84 was unable to interact with other golgins such as p115 and GM130 [10].

A second study [13] on golgin-84, using an *in vitro* assay for Golgi reassembly, found that addition of a soluble fragment comprising the Rab1-binding cytoplasmic domain of golgin-84 promoted lateral growth of Golgi cisternae, while having no other discernable effects. How exactly this effect was mediated is unclear, but the importance of golgin-84 for Golgi structure was further demonstrated by both overexpression of the protein and its depletion by RNA interference (RNAi); in both cases the Golgi 'ribbon' was fragmented [10]. In golgin-84-depleted cells, a decrease in Golgi membranes occurs that correlates with an increase in ER membranes, and a two-fold reduction in transport from the ER to the cell surface (Figure 1). This latter result is intriguing, as it suggests that transport through the cis-Golgi does not rely exclusively upon the p115–GM130 complex, and tallies with other recent work showing that GM130 can be dispensed with under certain conditions and is thus not as essential for Golgi structure and membrane transport as previously believed [14,15].

GM130 is a cis-Golgi-localised coiled-coil protein targeted to membranes via the peripheral membrane protein GRASP65 [16]. GM130 also associates with the Rab proteins Rab1, Rab2, and Rab33b, and with the vesicle-tethering protein p115 (Figure 2) [4]. This latter interaction is thought to be crucial for both vesicle docking at the cis-Golgi and for the post-mitotic reassembly of Golgi cisternae from vesiculated fragments. A causal relationship has been proposed between phosphorylation of GM130 by the Cdc2–cyclin B kinase [7], which disrupts the p115–GM130 interaction, and the vesiculation of the Golgi at the onset of mitosis (Figure 2).

This model is apparently contradicted by a recent study characterising the conditional lethal mutant cell line ldlG, which is deficient in expression of the low-density lipoprotein receptor at the plasma membrane [15]. Although ldlG cells contain no detectable GM130 at 34°C, the permissive temperature for growth, they nevertheless have an organised and functional Golgi apparatus [15]. This is not to say these cells are entirely normal, as they exhibit reduced levels of protein secretion at the non-permissive temperature of 39.5°C and eventually die. This temperature-sensitive phenotype corresponds to a reversible disassembly of the Golgi complex at 39.5°C, compared to a normal

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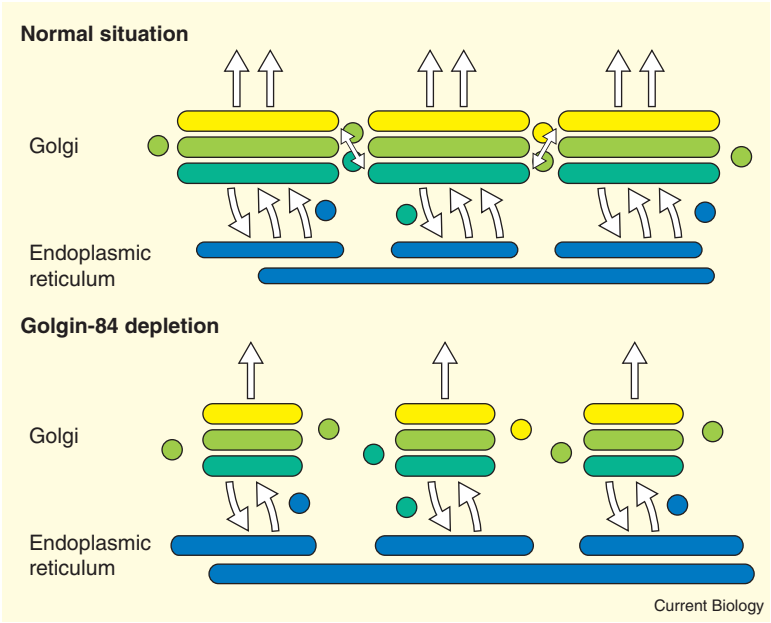

Figure 1. Golgin-84 and long-range Golgi organisation: ribbon formation.

Golgi ribbons are formed from Golgi stacks connected at their edges by dynamic tubular networks organised in the perinuclear region of animal cells adjacent to exit sites on the ER. The rate and extent of protein transport is a function of forward transport from ER to the Golgi, recycling pathways back to the ER, lateral exchange of material between stacks, and exit from the trans-Golgi network. In golgin-84-depleted cells, stereological analysis reveals that 50% of the Golgi membrane is relocated to the ER, and Golgi mini-stacks lacking this lateral organisation are present. These perturbations result in a reduction in the extent of protein transport by a factor of two.

Golgi structure at 34°C [15]. Importantly, the growth and secretion phenotypes of ldlG cells can be rescued by transfection to restore expression of GM130.

While the disruption of Golgi structure in ldlG cells at 39.5°C implies that GM130 is important, the normal operation of the Golgi at lower temperatures in its absence casts doubt on its previous placement as the central component [17]. This suggests the existence of additional mechanisms for vesicle tethering at the cis-Golgi, and golgin-84 is one obvious candidate molecule. The precise contributions of GM130, golgin-84 and p115 to cis-Golgi function still need to be elucidated, but given that the only common feature uncovered thus far is the interaction with Rab1, this seems to place this GTPase at the heart of both structural and protein transport pathways. This raises a somewhat problematic issue, as Rabs are thought to mediate specific membrane-tethering reactions [18], and this simple model does not appear to fit with the increasing number of effectors for Rab1, which at this stage can almost be described as promiscuous.

Do golgin-84 and GM130 have different roles in maintaining Golgi structure? Diao *et al.* [10] suggest that golgin-84 may be involved in the homotypic fusion of neighbouring Golgi stacks to form the Golgi ribbon, while GM130 may be more important for vesicle docking at the cis-Golgi. The inhibition of protein transport after golgin-84 depletion might therefore represent a decrease in the efficiency of transport through individual Golgi stacks, as opposed to through the more organised Golgi ribbon. Similarly, the absence of GM130 in ldlG cells affects Golgi organisation in a way that only becomes critical at 39.5°C, possibly because of heat stress or changes in membrane and cytoskeletal dynamics, while other factors such as golgin-84 may be sufficient at lower temperatures (Figure 2).

The findings of Vasile *et al.* [15] can be reconciled with previous data on the role of GM130 in the mitotic disassembly of the Golgi if the phosphorylation of additional Golgi proteins is needed *in vivo*. Many of the experiments on GM130 have been performed

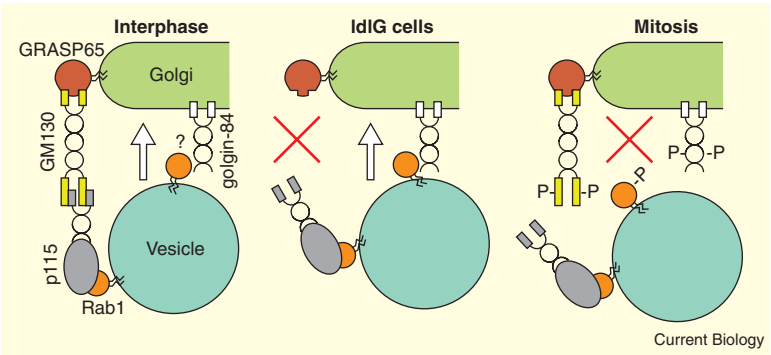

Figure 2. GM130 and golgin-84: vesicle tethering factors at the cis-Golgi.

During interphase, GM130 acts as a Golgi receptor for the vesicle-tethering factor p115, itself recruited to ER-derived vesicles and vesicular-tubular clusters by the Rab1 GTPase. In ldlG cells, which lack detectable levels of GM130, vesicle tethering at the cis-Golgi must rely on factors other than GM130. Golgin-84, which is also capable of binding Rab1 may be one such factor. Although these additional factors are sufficient to maintain normal Golgi structure and function in ldlG cells at 34°C, GM130 function appears to become essential at 39.5°C. A causal relationship has been proposed between GM130 phosphorylation in mitosis by the Cdc2–cyclin B kinase, which prevents p115 binding, and the block in ER-to-Golgi transport. The potential existence of additional tethering pathways, possibly involving golgin-84, raises questions about the link between GM130 phosphorylation and abolition of ER to Golgi transport in mitosis.

in vitro and it is possible that, under these conditions, the function of GM130 becomes essential, as it is in ldlG cells at 39.5°C. In this context it is worth noting that the assays for *in vitro* reassembly of the Golgi lack any of the cytoskeletal organisation present in living cells, and this could accentuate the contribution of factors such as GM130. The fact that golgin-84 and Rab1 are also phosphorylated during mitosis supports the idea that phosphorylation of GM130 is insufficient for mitotic Golgi breakdown *in vivo* (Figure 2).

At present, the precise function of golgin-84 phosphorylation is unclear; the obvious possibility that it regulates Rab1 binding remains untested, and a full answer to this question may require the identification of additional golgin-84 interactors. The conservation of histidine and tyrosine residues at fixed positions in the transmembrane domains of golgin-84, giantin and CASP indicates that interactions with other membrane proteins may be important for the functions of this particular group of Golgi proteins [9]. Analysis of the function of the yeast CASP homologue, COY1, has revealed these residues are critical for its correct localisation and function [9], and their conservation indicates this is likely to be true for the mammalian transmembrane golgins. Genetic interactions were observed between COY1 and key SNAREs, protein complexes that mediate membrane fusion events crucial for ER-to-Golgi traffic; while further studies are needed to identify interactions at the protein level, this does indicate that dynamic interactions with SNAREs could be the key to this problem.

An intriguing and perhaps key finding of Vasile *et al.* [15] was that the lack of GM130 in ldlG cells, together with the temperature-sensitive phenotype, could be reversed by overexpression of the N-ethylmaleimide sensitive factor (NSF), an ATPase essential for recycling SNARE complexes and hence membrane fusion and transport. While the increase in GM130 levels upon NSF overexpression may reflect a chaperone-like function resulting in stabilisation of GM130 protein, which is normally degraded in these mutant cells, this observation could in fact indicate a more complex functional relationship between GM130 and NSF. Both proteins associate directly or indirectly with SNARE proteins [19], and overexpression of NSF may alter the interaction between GM130 and the SNAREs, such that GM130 is stabilised and can thus carry out its normal function.

The multiplicity of interactions between golgins such as GM130 and golgin-84, Rab GTPases and other components of the membrane trafficking machinery may partly explain the different contributions these proteins make to Golgi structure. However, the precise nature and dynamics of these interactions must also be investigated before we can reach a fuller understanding of how golgins regulate Golgi function. The Golgi matrix still deserves the hypothesis tag, but with this in mind recent observations may represent not so much a glitch in the matrix as a glimpse into its true nature.

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